Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil

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Methanogenesis and digestibility in dairy cows fed linseed lipids

Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil[1]

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ABSTRACT: This experiment studied the effect of 3 forms of presentation of linseed fatty acids (FA) on methane output using the sulphur hexafluoride tracer technique, total tract digestibility, and performance of dairy cows. Eight multiparous lactating Holstein cows (initial milk yield 23.4 ± 2.2 kg/d) were assigned to 4 dietary treatments in a replicated 4 × 4 Latin square design: a control diet (C) consisting of corn silage (59%), grass hay (6%) and concentrate (35%), and the same diet with crude linseed (CLS), extruded linseed (ELS), or linseed oil (LSO) at the same FA level (5.7% of dietary DM). Each experimental period lasted 4 wk. All the forms of linseed FA significantly decreased daily CH_4 emissions ($P < 0.001$) but to different extents (-12% with CLS, -38% with ELS, -64% with LSO) compared with C. The same ranking among diets was observed for CH_4 output expressed as a percentage of energy intake ($P < 0.001$) or in grams per kilogram of OM intake ($P < 0.001$). Methane production per unit of digested NDF was similar for C, CLS, and ELS, but was lower for LSO (138 vs.

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68 g/kg digested NDF, respectively; $P < 0.001$). Measured as grams per kilogram of milk or fat corrected milk yield, methane emission was similar for C and CLS, and was lower for ELS and LSO ($P < 0.001$), LSO being lower than ELS ($P < 0.01$). Total tract NDF digestibility was significantly lower ($P < 0.001$) for the 3 supplemented diets than for C (-6.8% on average; $P < 0.001$). Starch digestibility was similar for all diets (mean 93.5%). Compared with C, DMI was not modified with CLS ($P > 0.05$) but was decreased with ELS and LSO (-3.1 and -5.1 kg/d, respectively; $P < 0.001$). Milk yield and milk fat content were similar for LSO and ELS but lower than for C and CLS (19.9 vs. 22.3 kg/d and 33.8 vs. 43.2 g/kg, on average, respectively; $P < 0.01$ and $P < 0.001$). Linseed FA offer a promising dietary means to depress ruminal methanogenesis. The form of presentation of linseed FA greatly influences methane output from dairy cows. The negative effects of linseed on milk production will need to be overcome if it is to be considered as a methane mitigation agent. Optimal conditions for the utilization of linseed FA in ruminant diets needs to be determined before recommending its use for the dairy industry.

Key Words: Dairy cows, Digestion, Fatty acids, Linseed oil, Linseeds, Methane

INTRODUCTION

A major concern of citizens in many countries today is the increased production of greenhouse gases and their impact on climate change. Methane (CH_4) is the second most problematic greenhouse gas after carbon dioxide (CO_2). Ruminant livestock are responsible for about 15 to 20% of the total anthropogenic emission of CH_4 (Moss et al., 2000). Methane emissions from ruminants also represent a loss of productive energy for the animal. Thus, the development of feeding strategies to mitigate these methane emissions may bring not only environmental benefits for the planet but also nutritional benefits for the animal. Dietary fatty

acids (**FA**), and more particularly PUFA**,** are among the most promising dietary alternatives able to depress ruminal methanogenesis (Martin et al., 2006). It has been shown that FA from linseed can decrease methane production in vitro (Broudiscou and Lassalas, 1991) as well as in vivo in sheep at maintenance (Czerkawski et al., 1966b) and in growing lambs (Machmüller et al., 2000). However, to our knowledge this effect has never been confirmed in dairy cows.

Linseed is not frequently used in ruminant feeding, especially because several experiments in which more than 5% linseed oil was supplied to sheep at maintenance have shown a strong negative effect on ruminal digestion (Ikwuegbu and Sutton, 1982). However, recent data have demonstrated that adding 3% linseed oil to dairy cows diets does not depress ruminal digestion (Ueda et al., 2003). Until now, no experiment has been conducted with dairy cows fed diets containing linseeds at levels above 3%. It is thus unclear whether the lack of negative effect of linseeds on digestion in dairy cows is due to the low level of supplementation. There is increasing interest in feeding linseed to dairy cows because of its FA profile; linolenic acid contributes dietary n-3 FA and promotes increased CLA content of milk from ruminants (Chilliard et al., 2007). Linseed oil was used in our study to examine the effects of linseed FA, but in practical feeding conditions, crude or extruded linseed would likely to be used as is more readily available, easy to use and less costly. Until now, no direct comparison of these 3 physical forms of linseed FA has been made using dairy cows.

The objectives of this trial were 1) to evaluate, in vivo, the effect of lipid supply from linseed on the emission of CH_4, and 2) to assess the consequences of a relatively high level of linseed supplementation on digestive efficiency and performance of dairy cows. Three diets containing crude linseed, extruded linseeds, and linseed oil plus linseed meal were compared to a control diet. Methane production, diet digestibility and performance of dairy cows were

71 determined, and the relationship between CH_4 production and dietary characteristics and milk
72 yield was evaluated.
73
74 **MATERIALS AND METHODS**
75
76 ***Animals, Experimental Design, and Diets***
77 Eight lactating multiparous Holstein cows (213 ± 40 days in milk) with an average milk
78 yield of 23.4 ± 2.2 kg/d and an average BW of 672 ± 54 kg at the beginning of the experiment
79 were used. Animals were blocked according to their physiological stage (4 non-pregnant cows
80 and 4 pregnant cows), and assigned to 4 dietary treatments in a replicated 4 × 4 Latin square
81 design. Each experimental period lasted 4 wk.
82 The treatments were 1) control diet (**C**), 2) diet C with crude linseed (**CLS**), 3) diet C
83 with extruded linseed (**ELS**), and 4) diet C with linseed oil (**LSO**). The control diet consisted
84 of 58.7% corn silage, 6.4% grass hay, and 34.9% concentrates, on a DM basis. Linseed oil
85 (Vandeputte Savonnerie et Huilerie, Mouscron, Belgium) was added to achieve a theoritical
86 oil level of 5% of dietary DM and replaced part of the concentrate portion of the basal diet to
87 obtain isoenergetic diets on an NE_L basis (target value of 7.1 MJ/kg DM). In the CLS and
88 ELS diets, proportions of crude and extruded linseed were calculated so that the mean oil
89 content of these diets was similar to that of the LSO diet. A level of 5% added lipids was
90 considered desirable to test the effects of lipids on rumen methanogenesis and to evaluate
91 differences due to form of linseed FA. Crude linseed was given as unprocessed whole seeds.
92 Extruded linseed (INZO, Château-Thierry, France) consisted of an extruded mixture of 70%
93 linseed and 30% wheat. After a short cooking period (5 min, 110°C, 3 atm), extrusion was
94 performed using a 1-screw extruder with an output temperature of 130°C. Incorporation of the
95 3 forms of linseed oil in the diets was achieved during a 3-d transition period. In addition, 200

g/d of a commercial mineral-vitamin premix (Galaphos Midi Duo GR, CCPA, Aurillac, France) was added to all diets. Ingredients and chemical composition of the experimental diets as consumed are given in Table 1. Diets were formulated according to meet the cow's requirements for maintenance and milk production (INRA, 1989). These requirements were calculated at the beginning of the experiment from milk yield at that time and were readjusted each experimental period assuming a monthly decrease in milk production of 10%. Diets were also formulated to contain the same quantity of limiting intestinal digestible protein (PDI system, INRA, 1989) supplied by all feedstuffs containing linseed (linseed meal, crude and extruded linseeds).

Forages (hay and corn silage) were offered once daily at 0900 with ad libitum access for corn silage (10% refusals). Concentrates were allocated separately from forages in 2 equal portions at 0900 and 1600 using a bucket to ensure complete consumption of the linseed. The forage:concentrate ratio was maintained as close as possible to the targeted ratio by adjusting the amounts of forages and concentrates offered daily based on the composition of the previous day's refusals. Crude and extruded linseed were mixed manually with the other concentrate ingredients immediately before feeding. Linseed oil was administered twice daily by drenching with the aid of a syringe. This way of distributing the oil was chosen because in a pre-experimental period mixing oil with the concentrate obstructed the capillary tube used for gas collection using the tracer technique.

Cows were kept in individual stalls in a well-ventilated shed to avoid accumulation of gases eructed by animals in ambient air, and had free access to water throughout the experiment. They were milked twice daily at 0630 and 1630. All experimental procedures were conducted in accordance with French guidelines for the use of experimental animals and animal welfare (Anonymous, 1988).

Measurements and Analyses

Intake and Milk Yield. Feed intake and orts were measured and recorded on 5 consecutive days each week throughout the experiment to calculate DMI. Dry matter content in feeds was measured at 60°C for 72 h every day for corn silage and once per week for other feeds. Dry feed samples were pooled at the end of each experimental period for corn silage and the end of the experiment for the other feeds. These samples were ground (0.8-mm screen) and analyzed for OM, N, NDF, ADF, starch, ether extract (EE), total FA, and GE. Fresh samples of each feed (1 kg for corn silage, 100 to 200 g for other feeds) were also taken at wk 4 and stored (-25°C for corn silage and 4°C for other feeds) before being pooled at the end of the experiment. These samples were freeze-dried, ground (0.8-mm screen), and analyzed for FA content.

Organic matter content of feeds was determined by ashing at 550°C for 6 h (AOAC, 1990). Nitrogen was analyzed by the Kjeldahl procedure (AOAC, 1990). The NDF and ADF contents were determined by sequential procedures (Van Soest et al., 1991) after pretreatment with amylase and were expressed inclusive of residual ash. Starch was analyzed using a polarimetric method (AFNOR, 1985). The GE content of feeds was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, Leics, UK). Determination of EE was performed according to AOAC (1990). Fatty acids from linseed oil were directly methylated with 2 mL of 0.5 *M* $NaOCH_3$ in methanol at room temperature for 20 min, followed by 1 mL of 5% HCl in methanol at room temperature for 20 min. Fatty acids in feedstuffs were extracted using a 2:1 chloroform-methanol mixture. Fatty acid methyl esters were recovered in 1 mL of hexane. Tricosanoate (Sigma, Saint-Quentin-Fallavier, France) was added as internal standard. Methyl esters were injected into a Trace-GC 2000 Series gas chromatograph equipped with a flame ionization detector (Thermofinnigan, Les Ulis, France). Methyl esters were separated using a fused silica capillary column (100 m ×

146 0.25 mm i.d.; CP-Sil 88, Chrompack, Middelburg, The Netherlands). Conditions for
147 chromatography analysis were as described in Loor et al. (2005).

148 Milk yield was determined on the same 5 consecutive days as for intake from wk 1 to
149 wk 4. On wk 4, milk samples were taken at each milking on d 2 and d 4. One 50-mL aliquot
150 of milk containing potassium bichromate (Merck, Fontenay-Sous-Bois, France) was stored at
151 4°C until analyzed for fat, protein, and lactose by infrared analysis with a 3-channel
152 spectrophotometer (AOAC, 1997). Milk energy was calculated from its fat, protein, and
153 lactose content (Tyrell and Reid, 1965).

154 ***Diet Digestibility.*** Total tract digestibility was determined from total collection of
155 feces for 5 d in wk 4. Feces were removed once daily for weighing and mixing before
156 sampling a 1% aliquot. After DM determination (60°C for 72 h), dry fecal samples were
157 pooled across days for each cow and each period, and then ground (0.8-mm screen) and
158 analyzed for OM, starch, NDF and ADF as described previously.

159 ***Methane Emissions.*** Methane production was determined during the same 5 d as for
160 digestibility in wk 4, using the sulphur hexafluoride (SF_6) tracer technique (Johnson et al.,
161 1994) as described by Pinares-Patiño et al. (2003). Brass permeation tubes (12.5 mm × 40 mm
162 i.d.) weighing about 32 g were used. These were loading with about 600 mg of SF_6 at liquid
163 N_2 temperature (-196°C) and calibrated by regular weighing (twice a week) for an 8-wk
164 period, while immersed in a water bath at 39°C. Permeation rate of SF_6 from the tubes was
165 1.523 ± 0.351 mg/d. A calibrated permeation tube was dosed per os into the rumen of each
166 cow 2 wk before sampling gas in period 1. Representative breath samples from each animal
167 were sampled in pre-evacuated (-0.9 atm) yoke-shaped PVC collection devices (~ 2.5 L) by
168 means of capillary and Teflon tubing fitted to a halter. The collection devices were changed
169 every 24 h before the morning feeding. The devices containing the samples were immediately
170 transported to the laboratory and over-pressured with N_2 gas to about 1.4 atm before SF_6 and

171 CH_4 analyses. Background concentrations of these gases were also measured in ambient air
172 samples collected every day in the shed during the same 5-d breath sampling period. Daily
173 CH_4 production from each animal was calculated according to Johnson et al. (1994), using the
174 known permeation rate of SF_6 and the concentrations (above the background) of SF_6 and CH_4
175 in the breath samples:

176 $$CH_4 \text{ (g/d)} = SF_6 \text{ permeation rate (g/d)} \times [CH_4]/[SF_6]$$

177 Concentrations of SF_6, and CH_4 in breath and ambient air samples were determined by
178 gas chromatography. A gas chromatograph (Varian-Chrompack, CP-9003, Les Ulis, France)
179 fitted with an electron capture detector (Perkin Elmer instruments; Autosystem XL,
180 Courtaboeuf, France) or with a flame ionization detector was used to determine the
181 concentrations of SF_6 and CH_4, respectively. The samples were run on chromatographs
182 equipped either with a Molecular Sieve 0.5 nm column (3 m × 3.2 mm i.d) maintained at 50°C
183 for the SF_6, or with a Porapak N 80-100 mesh column (3 m × 3.2 mm i.d.) maintained at 40°C
184 for the CH_4. The flow rate of the carrier gas was 30 mL/min of N_2 for the SF_6 and 40 mL/min
185 of He for the CH_4. Chromatographic analyses were performed after calibration with standard
186 gases (Air Liquide, Mitry-Mory, France) for SF_6 (55 and 195 ppt) and CH_4 (100 ppm).

187 ***Statistical Analyses.*** Data on CH_4 production, diet digestibility, DMI, and milk
188 production were averaged over the first 5 d of wk 4 before statistical analysis. All data from
189 the experiment were analyzed as a 4 × 4 Latin square using the MIXED procedure of SAS
190 (SAS Inst., Inc., Cary, NC). The statistical model included cow, period, treatment, and
191 residual error. Fixed effects included period and treatment. Cow was the random effect.
192 Overall differences between treatment means were considered to be significant when $P <$
193 0.05.

194

195 **RESULTS**

196

197 ***Feed Intake and Milk Production***

198 Feed intake parameters are presented in Table 2. Compared with diet C, diet CLS had
199 no effect on total DMI ($P > 0.05$), but diets ELS and LSO decreased total DMI (-3.1 and -
200 5.1 kg/d, respectively; $P < 0.001$), mainly through a decrease in corn silage intake (-2.7 kg/d
201 and -4.0 kg/d, respectively; $P < 0.001$). The negative effect on DMI was greater for LSO than
202 for ELS ($P < 0.01$). As a consequence, GE intake was significantly lower for LSO than for
203 ELS diet ($P < 0.01$), and lower for ELS than for CLS and C diets ($P < 0.001$).

204 Milk yield and 4% FCM yield were similar for the LSO and ELS diets, but these were
205 lower than the C and CLS diets (Table 2). Compared to diet C, milk fat content tended ($P =$
206 0.09) to be higher for CLS (+4.3 g/kg) but was lower ($P < 0.001$) for ELS (-5.8 g/kg) and
207 LSO (-8.8 g/kg). Protein and lactose contents did not vary among diets. Milk energy output
208 was 72.6 MJ/d on average for diets C and CLS, but was lower for diets ELS and LSO (-15.3
209 MJ/d on average; $P < 0.001$).

210

211 ***Diet Digestibility***

212 Dry matter and OM digestibilities were significantly lower ($P < 0.01$) for the 3
213 supplemented diets than for the C diet (-4.0 and -4.2 percentage units on average,
214 respectively, Table 3). This difference was due to a decrease in NDF digestibility ($P < 0.05$),
215 because starch digestibility was similar for all diets (93.5% on average). The decrease in NDF
216 digestibility was numerically greater for the ELS diet (-9.4 percentage units) than for the CLS
217 or LSO diets (-5.5 percentage units on average), but differences among the 3 supplemented
218 diets were not significant ($P > 0.1$). Digestibility of ADF was also lower for CLS and ELS
219 than for C and LSO diets ($P < 0.01$).

220

221 ***Methane Output***

222 Daily methane emissions differed ($P < 0.001$) amongst all the diets (Table 4). The
223 ranking of diets for daily methane production was C > CLS > ELS > LSO. The same ranking
224 was observed for CH_4 output reported as grams per kilogram of OM intake or as a percentage
225 of GE intake ($P < 0.001$). Methane output in grams per kilogram of NDF intake as well as in
226 grams per kilogram of digested OM was highest for C and CLS, intermediate for ELS, and
227 lowest for LSO ($P < 0.001$). Methane production per kilogram of digested NDF was similar
228 ($P > 0.05$) for C, CLS and ELS diets (138 g/kg digested NDF on average), but much lower for
229 the LSO diet (68 g/kg digested NDF). Methane production per kilogram of milk or FCM
230 produced was similar for C and CLS diets but lower for ELS and LSO diets, with the ELS diet
231 ranked higher than the LSO diet ($P < 0.001$). Energy lost as methane when expressed as a
232 percentage of milk energy output was similar for C, CLS, and ELS diets (28.7% of milk
233 energy on average) but was lower for the LSO diet (15.3% of milk energy, $P < 0.001$).
234
235 **DISCUSSION**
236
237 ***Feed Intake and Milk Yield***

238 The lack of effect of CLS on DMI is in agreement with previous findings (Ward et al.,
239 2002; Gonthier et al., 2005). A decrease in DMI with ELS or LSO was not observed in earlier
240 studies (Gonthier et al., 2005; Loor et al., 2005; Bu et al., 2007), except by Offer et al. (2001),
241 who used a diet based on corn silage, as in the present study. The decline in DMI that
242 occurred when LSO was fed cannot be fully explained by disturbances in rumen function,
243 because digestibility was not different among the 3 supplemented diets. It is possible that the
244 FA intake had a direct inhibitory effect on voluntary intake via inhibition of ruminoreticular
245 motility (Chilliard, 1993).

Dietary lipids generally increase milk yield as reviewed by Chilliard and Ferlay (2004). This increase has been reported specifically for linseed oil more (Bu et al., 2007) or less intensely (Loor et al., 2005), whereas a decrease in milk yield has been observed with extruded linseeds (Gonthier et al., 2005; Akraim et al., 2007). The decrease in milk and FCM yield and fat content observed in our study with both ELS and LSO diets, was probably caused by the lower DMI and the lower digestibility of fiber due to the high level of oil intake (5% of DMI). In addition, a lower mammary lipogenesis may have occurred as a result of adding polyunsaturated oil to a starch-rich diet (Chilliard et al., 2007). The lack of negative effect of feeding CLS on DMI, milk yield, and fat content, and 4% FCM, is likely due to the fact that CLS did not release FA in the rumen fluid as rapidly as ELS and LSO did, and thus rumen function was not disturbed.

Diet Digestibility

In this experiment, supplying 5.7% lipids from linseed significantly reduced OM and fiber digestibility of a corn silage-concentrate diet fed to dairy cows. This negative effect has been shown in sheep at maintenance receiving a supplement of 5% (Cottyn et al., 1971) or 7% (Ikbuegbu and Sutton, 1982; Sutton et al., 1983) linseed oil in hay-concentrate diets. By contrast, other experiments in dairy cows (3% linseed oil with either a hay-based diet, Ueda et al., 2003, or a corn silage-based diet, Ferlay and Chilliard, unpublished data) or dry cows (2.5% of FA from linseed or linseed oil, Doreau et al., unpublished data), in lambs (6.7% linseed, i.e., 2.5% FA, Machmüller et al., 2000) or in sheep (10.5% linseed, i.e., 4.8% FA given 12 times/d, Wachira et al., 2000), did not show any decrease in cell wall digestibility due to lipids from linseed. Furthermore, Gonthier et al. (2004) showed an increase in total digestibility of OM and fiber with a supplement of 3.5 to 4% FA from extruded linseed added to a grass and corn silage-based diet. From these experiments combined, it can be concluded

that the amount of added lipids and their form of presentation (oil vs. seed), are major determining factors for the negative effect of linseed FA on digestibility. Providing linseed twice daily in the present study may have contributed to a high decrease in digestibility, as the effects on digestibility have been less in a study where cows were fed 3 times daily a diet with 3% linseed oil (Ueda et al., 2003). In addition, we speculate that the negative effect of lipids on digestion is more pronounced with corn silage diets than with hay diets, based on results from our study and the study by Ben Salem et al. (1993) in which cows were fed a diet containing 7% rapeseed oil.

In ruminants, about 90% of total digestible fiber is digested in the rumen, although a possible decrease in ruminal fiber digestion can be partially compensated for by digestion in the large intestine. Thus, the 7 percentage unit decrease in NDF digestibility in the digestive tract observed in the present trial probably resulted from an even larger decrease in ruminal digestion (Ikwuegbu and Sutton, 1982; Sutton et al., 1983). Starch digestion was not altered by the 3 linseed FA supplements. This is consistent with previous data on different sources of lipids, in particular with linseed oil in cows (Ueda et al., 2003) and sheep (Ikwuegbu and Sutton, 1982) and linseed in lambs (Machmüller et al., 2000).

The absence of any differences in digestibility between CLS, ELS, and LSO diets was unexpected. It is generally thought that the inclusion of oil in seeds gives a partial protection against microbial attack or limits the effects of oil on ruminal microbes or both. For linseed, the present results suggest that linseed hulls did not prevent FA release in the rumen. Very few experiments have compared the effect of different forms of oleaginous seeds on digestion in ruminants. Gonthier et al. (2004), comparing crude and extruded linseed, found no evidence for any difference between forms, in agreement with the present experiment. A similar absence of difference between crude and extruded oleaginous seeds has been shown by others (Ferlay et al., 1992; Petit et al., 1997) with soybean or rapeseed. Only a few comparisons

296 between seeds and oils have been published. Pallister and Smithard (1987) reported a trend
297 towards a lower ruminal OM digestibility with extruded rapeseed than with crude rapeseed or
298 rapeseed oil, as observed in our study for fiber digestibility with ELS compared to CLS and
299 LSO (P = 0.11). Had we used more animals in our study, we might have detected the small
300 differences amongst linseed treatments. According to the literature and the present data, the
301 form of lipid supplementation does not seem to significantly modify diet digestibility, but
302 more research is needed to conclude on this point.
303
304 ***Methane Emissions***
305 Methane emission obtained for the control diet (418 g/d and 17.4 g/kg milk) are in
306 agreement with those reported in the literature (392 to 464 g/d and 14.3 to 19.6 g/kg milk)
307 with the tracer method (Lovett et al., 2005) and in respiratory chambers (Vermorel, 1995;
308 Sauer et al., 1998; Kinsman et al., 1995) for dairy cows at a similar level of milk production
309 (20 to 30 kg milk/d). In our experiment, cows lost 6.7% of GE intake as eructed methane with
310 the control diet, which was similar to values (6.2 to 6.7%) reported by Vermorel (1995) for
311 dairy cows of similar breed and physiological and nutritional conditions, and for small dairy
312 ruminants such as ewes and goats (6.2 to 6.3%).
313 Supply of lipids from linseed significantly decreased the amount of CH_4 emitted by
314 dairy cows, with a marked effect of the different forms of linseed FA (-12% with CLS, -38%
315 with ELS, -64% with LSO compared with the C diet). Thus, inhibition of the ruminant
316 methanogenesis may increase with the theoretical availability or release pattern of linseed FA
317 (LSO > ELS > CLS) in the rumen, whereas no such difference was observed for digestibility.
318 The decrease in methane emission with linseed oil in dairy cows confirms in vitro data
319 (Broudiscou and Lassalas, 1991). A depressive effect of linseed FA on in vivo CH_4 emissions,
320 quantified in respiratory chambers, has been shown in growing lambs supplemented with

6.7% of crushed whole linseed (i.e., 2.5% of oil; Machmüller et al., 2000) or in sheep at maintenance receiving 5% of linseed oil in intraruminal continuous infusion (Czerkawski et al., 1966a). In this last trial, the decrease in methane (-38%) was less than in the present study (-64%) with a similar level of linseed oil supplementation. However, the distribution pattern of oil differed between these 2 studies (continuous vs. twice daily). The negative effect of linseed oil FA on methanogenesis has been shown to be smaller when the same quantity of FA is distributed continuously compared with once (Czerkawski et al., 1966b).

The reduction in methanogenesis with added linseed FA cannot be explained by the reduction in intake. When methane emission is expressed per kg of OM or NDF intake, the same ranking between diets occurred in terms of their reduction in methane (LSO > ELS > CLS > C). However, when methane production was expressed per kg digested NDF, it was similar for C, CLS, and ELS diets but was lower for the LSO diet. Thus, the reduced fiber digestibility explained the decrease in methane production that occurred when diets were supplemented with CLS and ELS. The PUFA in free oil probably interact more rapidly with microorganisms in the rumen than FA in seeds. This is evidenced by a more pronounced shift of the VFA pattern towards propionate for oils than for seeds (review by Jouany et al., 2000). This effect may be emphasized by the mode of dispensing of the oil used in this study (twice daily by oral dosing) for the LSO diet. Thus a shift in fiber digestion from the rumen to the large intestine may have occurred for the LSO diet, and, as a consequence, less methane was produced per unit of digested NDF. The omission of the hindgut methane by the SF_6 technique probably resulted in an underestimation of methane production for the LSO diet compared to the other diets. We can assume that differences among diets in fiber digested in the rumen are higher than differences in the total tract. This has been shown by Sutton et al. (1983), who observed a larger decrease in OM digestion in the rumen (-19 points) than in the total tract (-3 points) in sheep supplemented with 7% linseed oil. Thus, had fiber digestion in

346 the rumen been measured, it may have explained the differences in methanogenesis between
347 the 3 diets containing FA from linseed.

348 Polyunsaturated FA decrease methane through a toxic effect on microorganisms
349 involved in fiber digestion and hydrogen production such as protozoa (Doreau and Ferlay,
350 1995) and cellulolytic bacteria (Nagaraja et al., 1997). This effect, observed with all long-
351 chain FA, is probably through an action on the cell membrane particularly of Gram-positive
352 bacteria. It has been shown in vitro that linolenic acid is particularly toxic for the 3 cellulolytic
353 bacterial species (*Fibrobacter succinogenes, Ruminococcus albus*, and *R. flavefaciens*) as it
354 disrupts cell integrity (Maia et al., 2006). In addition, a direct toxic effect of PUFA on
355 methanogens that use hydrogen for methane production may have occurred, as shown in vitro
356 with linseed oil hydrolysate (Prins et al., 1972). In this case, free hydrogen may accumulate in
357 the gas mixture, resulting in growth inhibition of cellulolytic bacteria (Wolin et al., 1997), and
358 fiber digestibility may be impaired as observed in the present experiment.

359 The effects of FA from linseed on methanogenesis were observed in our study for
360 cows fed the different diets for 4 wk, but these results need to be confirmed in a longer-term
361 study. An adaptation of the rumen microflora to oil supplementation over the long term may
362 be possible and the long-term persistence of methane suppressing feed manipulations has been
363 recognized as an important issue (Woodward et al., 2006; Grainger et al., 2008).

364 This study demonstrates that a 5.7% supply of lipids from linseed significantly
365 decreases the quantity of CH_4 emitted daily by dairy cows, with a marked effect of the
366 physical form of linseed FA. Inhibition of rumen methanogenesis appears to increase with the
367 theoretical availability of linseed FA in the rumen. The use of linseeds in dairy cows diets
368 may result in positive environmental effects. However, their use as a mitigating agent requires
369 sustained long-terms effect on methane without causing negative effects on animal
370 performance. Impact of the different forms of linseeds or oil on milk quality in terms of FA

371 profiles (increase in n-3 FA, CLA, trans-FA, etc.) also needs to be assessed. Optimal
372 conditions for the utilization of linseed FA in ruminant nutrition thus remains to be
373 determined before recommending their use in commercial dairy production. Further work
374 should consider lower levels of linseed supply, the form of adding the linseed lipids to the diet
375 (distribution pattern, variations in processing techniques), and the interaction with the nature
376 of the basal diet (pasture, grass silage, hay, or corn silage).
377

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Table 1. Ingredient and chemical composition of the experimental diets as consumed

	Diet [1]				
Ingredient, % of DM	C	CLS	ELS	LSO	SEM
Corn silage	58.7	59.6	54.1	51.3	1.09
Grass hay	6.4	6.7[c]	7.8	8.9	0.23
Concentrates	34.9	33.8	38.2	39.8	0.94
Concentrate mixture[2]	11.5	1.9	8.8	4.2	0.44
Extruded wheat	5.2	5.5	0.0	6.9	0.19
Soybean meal	7.6	8.3	8.1	8.6	0.29
Linseed meal	10.6	5.7	0.0	14.3	0.39
Crude linseed	0.0	12.4	0.0	0.0	0.19
Extruded linseed + wheat	0.0	0.0	21.2	0.0	0.37
Linseed oil	0.0	0.0	0.0	5.8	0.16
Mineral-vitamin mix[3]	1.0	1.0	1.2	1.4	0.02
Chemical composition					
OM, % of DM	95.3	95.5	95.3	89.9	0.15
CP, % of DM	14.5	14.9	14.6	14.6	0.19
NDF, % of DM	32.9	32.0	30.8	31.4	0.16
ADF, % of DM	17.5	16.9	16.7	16.6	0.10
Starch, % of DM	26.5	24.8	21.2	23.2	0.30
Ether extract, % of DM	2.6	6.8	7.0	8.4	0.16
GE, MJ/kg of DM	17.4	18.4	18.0	18.8	0.04
Fatty acid profile, % of total fatty acids					
14:0	0.39	0.20	0.16	0.15	0.003
16:0	15.15	9.74	8.61	8.17	0.065
18:0	2.49	2.83	2.88	2.49	0.073
18:1*cis*-9	19.85	16.43	15.45	15.09	0.040
18:1*trans*-11	0.91	0.67	0.64	0.70	0.066
18:2*cis*-9,*cis*-12	41.34	27.70	24.21	21.32	0.193
20:0	0.38	0.24	0.10	0.11	0.002
18:3*cis*-9,*cis*-12,*cis*-15	16.20	40.34	46.40	49.15	0.297
22:0	0.28	0.18	0.14	0.08	0.002
24:0	0.31	0.18	0.15	0.71	0.006
Others	2.52	1.39	1.18	1.09	0.023

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.
[2]Composition (g/kg): dehydrated beet pulp, 300; wheat, 200; barley, 200; rapeseed meal, 150; soybean meal, 70; beet molasses, 50; limestone, 10; dicalcium phosphate, 10; magnesium oxide, 5; sodium chloride, 5.
[3]Composition (g/kg): Ca, 200; P, 45; Mg, 45; Na, 50; Cu, 1.3; Zn, 6.0; Mn, 3.5; I, 0.08; Co, 0.032; Se, 0.020; vitamin A, 600,000 IU; vitamin D3, 120,000 IU; vitamin E, 1,300 IU.

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Table 2. Intake and milk yield and composition for lactating dairy cows fed diets supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	$P <$
DMI, kg/d						
Total	19.8[a]	19.5[a]	16.7[b]	14.7[c]	0.30	0.001
Silage	11.7[a]	11.7[a]	9.0[b]	7.7[c]	0.29	0.001
Concentrate	6.8[a]	6.6[a]	6.4[a]	5.8[b]	0.15	0.001
OMI, kg/d	18.9[a]	18.7[a]	15.9[b]	14.2[c]	0.28	0.001
GE intake, MJ/d	344.2[a]	358.1[a]	299.9[b]	275.8[c]	5.32	0.001
Milk yield, kg/d	23.0[a]	21.5[a]	20.8[ab]	18.9[b]	0.71	0.01
4% FCM, kg/d	23.4[a]	23.1[a]	18.9[b]	16.9[b]	0.77	0.001
Milk composition, g/kg						
Fat	41.1[a]	45.4[a]	35.3[b]	32.3[b]	1.71	0.001
Protein	34.0	34.6	33.3	34.7	0.67	NS[2]
Lactose	48.3	48.2	48.0	48.6	0.25	NS
Milk energy output, MJ/d	73.4[a]	71.7[a]	60.0[b]	54.6[b]	2.31	0.001

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.
[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).
[2]NS: not significant ($P > 0.05$).

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Table 3. Total-tract digestibility of DM, OM, fiber, and starch in lactating dairy cows fed diets supplemented with linseed

	Diet[1]					
Item	C	CLS	ELS	LSO	SEM	*P* <
DM, %	66.5[a]	62.2[b]	63.5[b]	61.7[b]	0.78	0.01
OM, %	70.0[a]	65.2[b]	66.7[b]	65.4[b]	0.78	0.01
NDF, %	47.5[a]	41.9[b]	38.1[b]	42.2[b]	1.74	0.05
ADF, %	44.7[a]	36.8[b]	34.1[b]	44.0[a]	2.22	0.01
Starch, %	93.4	93.0	93.0	94.7	0.54	NS[2]

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[2]NS: not significant ($P > 0.05$).

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Table 4. Methane emissions in lactating in lactating dairy cows fed diets supplemented with linseed.

	Diet[1]				SEM	$P <$
Item	C	CLS	ELS	LSO		
CH_4, g/d	418.1[a]	369.4[b]	258.1[c]	149.2[d]	13.64	0.001
CH_4, % GE intake	6.7[a]	5.7[b]	4.8[c]	3.0[d]	0.21	0.001
CH_4, g/kg OM intake	22.0[a]	19.8[b]	16.3[c]	10.5[d]	0.72	0.001
CH_4, g/kg NDF intake	63.8[a]	59.3[a]	50.7[b]	27.5[c]	2.19	0.001
CH_4, g/kg digested OM	31.4[a]	30.2[a]	24.5[b]	16.2[c]	1.08	0.001
CH_4, g/kg digested NDF	136.2[a]	141.0[a]	135.9[a]	68.1[b]	6.42	0.001
CH_4, g/kg milk	17.4[a]	17.9[a]	12.2[b]	8.1[c]	0.94	0.001
CH_4, g/kg 4% FCM	19.3[a]	16.4[ab]	14.8[b]	9.3[c]	1.27	0.001
CH_4, % milk energy output	33.8[a]	29.0[a]	25.7[a]	15.7[b]	2.30	0.001

[1]C = control; CLS = diet C including crude linseed; ELS = diet C including extruded linseed; LSO = diet C including linseed oil.

[a,b,c,d]Within a row, means without a common superscript differ ($P < 0.05$).

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